

PB-215 390

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ANAEROBIC WASTE TREATMENT. II: DAILY
FEED STUDIES

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New York University
New York, New York

1964

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CATION TOXICITY AND STIMULATION IN
ANAEROBIC WASTE TREATMENT

II
DAILY FEED STUDIES

by

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Presented on May 6, 1964 at the 19th Annual
Purdue Industrial Waste Treatment Conference
Lafayette, Indiana

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INTRODUCTION

During the past twenty years research into the fundamental principles underlying waste water treatment has increased at a rapid rate. One fundamental principle which this research has brought forth is that optimum efficiency in a biological waste treatment system can be attained only when the microorganisms functioning in the process are provided with an optimum environment. Studies by biologists over the last century have indicated the fundamental role played by cations in the metabolic processes of all organisms. Therefore, it is evident that control of the ionic environment must be practiced in order to insure optimum performance of biological waste treatment systems.

Until recently, however, the importance of cation effects has been overlooked in the design of anaerobic waste treatment systems. The neglect of cation effects has resulted primarily for three reasons:

1. In the past most anaerobic systems have been called upon to treat domestic sewage sludge. In most cases sewage sludge is essentially a biological well balanced ionic medium.
2. The anaerobic digestion systems designed for the treatment of domestic sewage sludge were operated at rather low organic loadings, and long retention times. Thus deviations from the optimum ionic environment were not critical.
3. Treatment difficulties which resulted from an ionic imbalance were ascribed to improper levels of other more easily recognizable environmental factors such as pH, and volatile acids.

In the future, biological waste treatment systems will be called upon more to handle wastes of an entirely different nature from that of domestic sewage sludge. In addition, in order to make the process as economical as possible, much higher organic loading rates and shorter retention times will be desirable. Under these new conditions consideration of cation effects will undoubtedly become more critical for the design and operation of anaerobic waste treatment systems.

In order to prepare for these future conditions, a basic investigation of cation effects in the anaerobic waste treatment process was undertaken. The first phase of this investigation dealt with the slug addition of cations to active cultures of acetate fermenting methane bacteria. The results of the first phase were reported previously (1). The second phase which is reported in this paper deals with cation effects under daily feed conditions, as would apply more closely to field conditions.

PREVIOUS WORK

In the first article of this series, the response of acetate fermenting methane bacteria to slug additions of sodium, ammonium, potassium, calcium and magnesium, both singly and in combination, was reported (1). The studies were carried out in a chemically defined growth medium low in organic and inorganic growth factors. The special medium was used to overcome difficulties in interpretation of results which would result if a non-chemically defined medium such as sewage sludge was used. It was found that the order of increasing toxicity on a molar basis was sodium, ammonium, potassium, calcium and magnesium. When these ions were present in low concentration, they produced stimulation rather than toxicity. Peak stimulation of acetate fermentation was achieved when the concentration of each monovalent cation was 0.01 M and when the concentration of each divalent cation was 0.005 M.

It was found that when mixtures of the cations were present, the phenomena of antagonism and synergism were manifest. Antagonism is the situation where the presence of one cation (the antagonist) reduces or eliminates the toxic effect of another cation. Synergism is the opposite situation. Here the addition of a second cation to a solution containing a toxic concentration of another cation produces an increase in the toxic effect. Cations which formed antagonistic and synergistic pairs are shown in Table 1.

Table 1

Cation Pairs Showing Antagonistic & Synergistic Combinations

Toxic Cation	<u>Na</u>	<u>NH₄</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>
Antagonists	K	Na	Na NH ₄ Ca Mg	Na K	Na K
Toxic Cation	<u>Na</u>	<u>NH₄</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>
Synergists	NH ₄ Ca Mg	K Ca Mg	—	NH ₄ Mg	NH ₄ Ca

A review of the significant biological and sanitary engineering literature on the nature of cation effects was presented in the first article of this series and will not be repeated here.

Most of the previous work on cation effects has been carried out on a slug addition basis in which a sudden addition of a cation or cation combination is made to an active bacterial population. While such a situation could arise in the operation of a waste treatment system, it is more likely that high concentrations of some cations will always be present in the waste. Thus, a more general situation for investigation would be one where cations are added daily with the waste substrate to a continuously operating treatment system.

When microorganisms are continuously in contact with a relatively high concentration of a cation or cation combination, the process of acclimation can come into play. Thus the results obtained in slug feed studies might be altered under continuous exposure conditions.

Studies of acclimation have been conducted by many biologists. MacLeod (2) reported on the acclimation of three species of lactic acid bacteria to zinc and lithium toxicity. Severens and Tanner (3) raised the tolerance level of S. pullorum, E. typhosa and S. schotmulleri to sodium chloride, copper sulfate and mercuric chloride. The tolerance level was raised from three to twelve times. Borman (4) acclimated two strains of E. coli to magnesium and mercury. He found that acclimation to either of the metals conferred acclimation to the other as well. In addition, acclimation to either of these cations raised the resistance of E. coli to sodium, potassium, iron and copper.

In the sanitary engineering field only scattered references on acclimation to cation toxicity are available. Rudolphs (5) reported initial retardation of digestion of sewage sludge by 5 g/l of sodium chloride. After a few days, however, digestion proceeded satisfactorily. Brosseau (6) also reported acclimation to sodium toxicity in a daily feed laboratory scale sewage sludge digester. Initially a 4 g/l concentration of sodium ion produced marked inhibition, but after acclimation digestion proceeded well at a sodium level of 5 g/l.

McCarty and McKinney (7) reported on the acclimation of acetate utilizing methane bacteria to a variety of cations. They found that in systems operating at a thirty day detention time and at an organic loading of two grams per liter per day the following individual cation concentrations could be acclimated to: Sodium = Potassium = 0.2 M, Calcium = Magnesium = 0.175 M, Ammonium = 0.15 M.

Despite the limited work on acclimation in the sanitary engineering literature it is clear that this phenomenon can have a significant bearing on the allowable cation concentration in a waste treatment system. The main purpose of the investigation reported here was to evaluate the possible extent of acclimation to the toxic effects of cations in an anaerobic waste treatment system. In addition, the studies were designed to spot test whether the relationships established in the slug feed studies (1) would hold on a daily feed schedule.

EXPERIMENTAL PROCEDURES

a) Growth Medium

The characteristics of the inorganic portion of the synthetic waste medium used in these studies is shown in Tables 2 and 3. This medium is the same as that used for the slug feed studies (1). Acetic acid was used as the organic substrate for biological decomposition and growth, and was added to this medium.

Table 2

Inorganic Portion of Growth Medium Utilized for Study of Cation Effects on Acetate Utilizing Methane Bacteria

<u>Substance</u>	<u>Quantity</u>
Cambridge Tap Water	1000 ml
Bicarbonate Buffer	2500 mg/l as CaCO_3
FeCl_3	60 mg/l
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	8 mg/l
Thiamine - HCl	2 mg/l
K_2SO_4	10 mg/l
NH_4Cl	147 mg/l
$(\text{NH}_4)_2 \text{HPO}_4$	22 mg/l

Table 3

Ionic Characteristics of Cambridge Tap Water

<u>Ion</u>	<u>Concentration mg/l</u>
Sodium	10
Potassium	1.8
Ammonium	less than 1.0
Magnesium plus Calcium	50-60 as CaCO_3

This medium contains sufficient inorganic nutrients to permit satisfactory operation of the laboratory units at an acetic acid loading rate to the digesters of 0.5 grams per liter per day at a 15 day detention time. The buffer level maintained the pH at 7.0. In the last run of this study the organic loading was raised to 1.0 gram per liter per day. In order to operate the digesters at the higher loading rate, some slight modifications in the medium were necessary. These modifications will be detailed later.

The only constituent of the medium which is present in high concentration is the cation associated with the bicarbonate buffer.

In order to eliminate any interference from the buffer cation, the cation which served as the major or toxic cation in any run also served as the buffer cation.

b) Description of Equipment

All studies were carried out in laboratory scale digesters constructed from 1 liter pyrex bottles filled to the 0.75 liter mark. Figure 1 illustrates a typical unit and its appurtenances. A description of this equipment has been presented previously (8).

c) Operation of Daily Feed System

All digesters were initially seeded with digested sludge obtained from the Nut Island Sewage Treatment Plant, Boston, Massachusetts. The units were operated on a once daily feed and withdrawal schedule. In the first four runs of the study, the organic loading used was 0.5 grams per liter per day while 1 gram per liter per day was the organic loading used in the fifth run. In all runs the hydraulic and biological solids retention time was 15 days. The units were operated for three months prior to the beginning of each run. During this period the initial seed material was practically all replaced by the growth medium detailed in Tables 2 and 3.

The only organic substrates fed to the units were acetic acid. Thus only acetate utilizing methane bacteria were active in the units. The acetate utiliziers were studied because previous studies have shown that these are the predominant group of methane organisms active in the degradation of almost all wastes (9).

d) Method of Cation Addition to the Units

The cations under study were all introduced in the form of chloride salts. Chloride salts were used because of their high solubility, and because the chloride anion is relatively non-toxic to methane bacteria. Standard solutions of the chlorides were prepared, and appropriate quantities were added to the daily feed. The daily feed thus contained the various constituents of the growth medium, the acetic acid and appropriate quantities of the chlorides of the cations under study. All solutions used to make up the daily feed were prepared in Cambridge Tap Water.

The procedures outlined above were established so that the effects of the cations could be ascertained:

1. Under digester operation procedures similar to those used in actual field installations,

2. In a medium as free as possible from factors other than the cations under study,

3. With cation introduction and build-up similar to what would occur in a field unit treating a waste with a high cation concentration.

e) Method of Calculation of Acetic Acid Utilization Efficiency

The following equation was used to determine the efficiency of acetic acid utilization in these studies:

$$E = \frac{I - O}{I} \times 100$$

where:

E = efficiency of acetic acid utilization in percent
 I = concentration of acetic acid in the feed in mg/l
 O = concentration of acetic acid in the effluent in mg/l

In the first four runs of this study the organic loading was 0.5 grams per liter of digester capacity per day at a 15 day detention time. Under these conditions, I is equal to 7,500 mg/l. In the last run of this study, the organic loading was raised to 1.0 grams per liter of digester capacity per day, while the detention time remained at 15 days. Under this condition, I equals 15,000 mg/l.

RESULTS

The first four runs of this study were conducted in order to:

1. Evaluate the extent of acclimation to the toxic effect of a single cation.
2. To determine the applicability of results obtained in the slug feed studies to daily feed operation.

In each of these runs a different cation was chosen as the toxic cation. The concentration of this cation was maintained at a high level in the feed to all units, except the control, from the first day of the run to its completion. Several times during the run the concentration of the toxic cation in the feed solution was increased. This procedure produced a gradual steady increase in the toxic cation concentration in the units during the run.

Combinations of cations were also studied in some runs to evaluate any antagonistic or synergistic effects which might result. Here, appropriate concentrations of cations which were tested for their antagonistic or synergistic ability were introduced to the units on the first day of the run. The levels of the antagonists or synergists were maintained at this initial concentration throughout each run.

Run 1 - Sodium Toxicity Daily Feed

Five units with one serving as a control were utilized in this run. The salts fed to the units, and the sodium chloride feed schedule are given in Tables 4 and 5. The sodium concentration in the control was 0.05 M, which is equal to that required to maintain the bicarbonate buffer at the appropriate concentration.

Table 4

Salts Fed to Units During Run 1

<u>Unit</u>	<u>Salts</u>
1	Control (none)
2	NaCl
3	NaCl + 0.01 M KCl
4	NaCl + 0.01 M NH_4Cl
5	NaCl + 0.01 M KCl + 0.01 M NH_4Cl

Table 5

Sodium Feed Schedule During Run 1

<u>Sodium Concentration in Feed</u>	<u>Time Period</u>
0.3 M	Day 1-16
0.4 M	Day 17-30
0.5 M	Day 31-33

In Figure 2 the results of this run are shown graphically. In this figure the effluent volatile acids concentration is plotted versus the sodium concentration. An alternate scale showing the number of days from the start of the run is also provided. These results show a very slow increase in sodium inhibition once the sodium concentration increased beyond 0.2 M. However, the unit still exhibited good operation at the conclusion of the run (efficiency of acetate removal of 84%), at which time the sodium concentration had reached 0.35 M. In the previous study (1) a slug dose of 0.35 M sodium reduced the efficiency of operation to 25% of the control. Thus a considerable degree of acclimation is evidenced here.

The antagonistic relationships observed in this run are in line with those obtained in the slug feed runs. The presence of 0.01 M potassium retarded the onset of any inhibition until the sodium reached 0.27 M. In addition, the magnitude of the inhibition produced beyond this point was markedly reduced. At the conclusion of

the run this unit exhibited an efficiency of acetate removal of 92% while the control efficiency was 94.5%.

Ammonium was found not to be an antagonist of sodium nor did its presence effect the ability of potassium to act as an antagonist. These results are in agreement with the slug feed studies. At concentrations greater than 0.01 M, ammonium was found to be a synergist of sodium in the slug feed studies. As this concentration of ammonium was not exceeded in this study, no synergism was expected, nor was any observed.

Run 2 - Potassium Toxicity Daily Feed

The salts fed to the units in this run and the potassium chloride feed schedule are shown in Tables 6 and 7. The potassium concentration in the control was 0.05 M throughout the test.

Table 6

Salts Fed to Units in Run 2

<u>Unit</u>	<u>Salts</u>
1	Control (none)
2	KCl
3	KCl + 0.01 M NaCl
4	KCl + 0.005 M CaCl ₂
5	KCl + 0.01 M NaCl + 0.005 M CaCl ₂

Table 7

Potassium Feed Schedule Run 2

<u>Potassium Concentration in Feed</u>	<u>Time Period</u>
0.3 M	Day 1-20
0.4 M	Day 21-28
0.5 M	Day 29-33

The results of run 2 are shown graphically in Figure 3. Inhibition by potassium first became evident at 0.13 M and by the time the concentration reached 0.25 M complete failure of the unit resulted. The actual cause of the complete failure of the unit was a low pH condition, which resulted from the reduced efficiency of acetic acid utilization caused by the potassium inhibition.

A comparison between the results of runs 1 and 2 indicates that potassium is more toxic than sodium which confirms the results of the slug feed studies. Despite the rather rapid failure of the potassium unit, acclimation was evident in that the efficiency of acetate utilization in the daily feed unit was 90% versus 60% in the slug feed unit (1) when the potassium concentration was 0.13 M.

Both sodium and calcium were found to be antagonists of potassium toxicity in the slug feed studies, with sodium producing slightly superior results. The results of the daily feed studies shown in Figure 3 confirm these results; however, here the sodium was much superior to calcium as an antagonist. Calcium did not delay the onset of potassium inhibition but the rate of increase in inhibition as the potassium concentration went beyond 0.13 M was reduced. With sodium present potassium inhibition was delayed until the potassium concentration exceeded 0.3 M. Even at 0.35 M potassium, the efficiency of acetate utilization with sodium present was quite good (89%).

The most striking result of this run was the excellent antagonism achieved by a combination of sodium and calcium. In the slug feed study it was also found that antagonism of potassium by sodium plus calcium was superior to that achieved by either one alone. However, the magnitude of the effect was much greater here. In fact, throughout the whole of this run the unit containing sodium plus calcium exhibited an efficiency of acetate utilization equal to or superior to that of the control. Even with the potassium concentration at 0.35 M the unit containing the combination of antagonists showed an efficiency of 96%, while the efficiency of the control was only 93.5%. This is a case of stimulation by the antagonistic cations, which was observed for several situations in the slug feed studies.

Run 3 - Magnesium Toxicity Daily Feed

Tables 8 and 9 give the salts fed to the units, and the magnesium chloride feed schedule during run 3. The magnesium concentration in the control was 0.025 M.

Table 8

Salts Fed to Units During Run 3

<u>Unit</u>	<u>Salts</u>
1	Control (none)
2	MgCl ₂
3	MgCl ₂ + 0.01 M NaCl
4	MgCl ₂ + 0.01 M KCl
5	MgCl ₂ + 0.01 M NaCl + 0.01 M KCl

Table 9

Magnesium Feed Schedule During Run 3

<u>Magnesium Concentration in Feed</u>	<u>Time Period</u>
0.1 <u>M</u>	Day 1-16
0.15 <u>M</u>	Day 17-30
0.20 <u>M</u>	Day 31-33

The results of this run are illustrated in Figure 4. Acclimation is again evidenced in this run as inhibition did not begin until the magnesium concentration reached 0.06 M. In the slug feed studies this concentration of magnesium reduced the reaction rate to 70% of the control. Complete failure of the unit fed only magnesium was attained at a magnesium concentration of 0.1 M. As in the case of the potassium run, the direct cause of total failure was a low pH resulting from the retardation of the rate of acetic acid utilization, rather than a complete stoppage of digestion directly by the magnesium.

The antagonistic effects observed in this run are in agreement with the results obtained in the slug feed studies. Both sodium and potassium individually antagonized magnesium toxicity, with sodium producing superior results. Potassium delayed the onset of inhibition until a magnesium concentration of 0.09 M, while sodium delayed inhibition until the magnesium concentration reached 0.11 M. As in the slug feed studies a combination of sodium plus potassium produced results superior to that produced by either cation alone.

Run 4 - Calcium Toxicity Daily Feed

The salts fed and the calcium feed schedule during this run are shown in Tables 10 and 11 respectively. The calcium concentration in the control was 0.025 M.

Table 10

Salts Fed to Units During Run 4

<u>Unit</u>	<u>Salts</u>
1	Control (none)
2	CaCl ₂
3	CaCl ₂ + 0.01 <u>M</u> NaCl
4	CaCl ₂ + 0.01 <u>M</u> NaCl + 0.005 <u>M</u> KCl
5	CaCl ₂ + 0.01 <u>M</u> NaCl + 0.005 <u>M</u> MgCl ₂

Table 11

Calcium Feed Schedule Run 4

<u>Calcium Concentration in Feed</u>	<u>Time Period</u>
0.15 M	Day 1-15
0.20 M	Day 16-27
0.30 M	Day 28-31

The results of this run are shown plotted in Figure 5. These results indicate that a significant amount of acclimation to the toxic effect of calcium was achieved. Only a slight inhibition resulted at a calcium concentration of 0.15 M and at the highest concentration attained (0.2 M), the unit exhibited satisfactory operation (acetate removal efficiency 94%). In the slug feed studies 0.2 M calcium completely stopped acetate utilization.

It is possible that the acclimation observed here was more apparent than real because some of the calcium added to the unit did not remain in solution. On the twenty-fifth day of the study the alkalinity in the unit was 1500 mg/l as CaCO_3 indicating a precipitation of calcium as calcium carbonate. A large quantity of a white precipitate built up on the walls of the unit as the calcium level increased, which gives a further indication of the precipitation of calcium. However, the quantity of calcium precipitating could not exceed 0.025 M, the concentration associated with the bicarbonate buffer. The remainder was in the form of the soluble calcium chloride.

Significant antagonism was achieved by the sodium ion in this run. All units containing sodium alone or in combination with potassium or magnesium showed operation superior to the unit containing calcium alone. In addition, all units containing sodium performed better than the control unit indicating stimulation had taken place. The combinations of sodium plus potassium and sodium plus magnesium produced results superior to sodium alone. The achievement of superior antagonism with two or more antagonists present was also observed in run 2 of this study and in the slug feed studies.

In addition, the results of this run illustrate the phenomenon of secondary antagonism which was observed in several of the slug feed runs. Sodium was found to be an antagonist of calcium in the slug feed study while magnesium was not. However, in the presence of sodium, the secondary cation magnesium can serve as an antagonist of calcium.

Run 5 - Effect of Long Term Exposure to Sodium

In the four previous runs the concentration of the toxic cation was raised continually to force the units toward failure. This procedure was adopted in order to illustrate differences between the antagonistic action of different cations and cation combinations. This is a slight departure from the situation which would exist in a field unit treating a waste with a constant cation concentration. In the latter situation the rate of increase of the toxic cation concentration would decrease with time and eventually the concentration in the unit would equal the concentration in the waste. This run was conducted to evaluate the operation of a unit under long time continuous exposure to a single level of a cation concentration.

Five units were established in this study with sodium serving as the buffer cation. The organic loading used was one gram per liter per day of acetate. In order to operate at this organic loading it was found necessary to make the following changes in the growth medium: the ferric chloride was raised to 100 mg/l, the thiamine was raised to 10 mg/l, the ammonium chloride was raised to 324 mg/l, the ammonium monohydrogen phosphate was raised to 44 mg/l, and both pantothenic acid and nicotinimide were added to the level of 20 mg/l. A different sodium concentration was used in each unit of this study. On the first day of the run, the sodium concentration in the feed was raised to the values listed in Table 12, and maintained at these levels throughout the remainder of the study. The sodium concentration maintained in the control was 0.05 M (1.15 g/l) which was necessary to keep the alkalinity at 2500 mg/l.

Table 12

Sodium Concentration in Feed Run 5

<u>Unit</u>	<u>mg/l</u>	<u>Molar</u>
1 (control)	1,150	0.05
2	2,000	0.087
3	3,000	0.13
4	4,000	0.174
5	6,000	0.26

The results of this study, presented graphically in Figure 6, show a gradual increase in effluent volatile acids in all units except the control during the early part of the run. However, once the sodium concentration in each unit leveled off, the operation of each unit reached equilibrium. After 45 days of operation (3 detention times) it can be assumed that the sodium concentration in the feed and the units was practically identical. It can be seen that

the higher the sodium concentration, the lower the efficiency of acetate utilization. Even the unit containing the low concentration of 2000 mg/l showed a very slight but definite decrease in efficiency with respect to the control. These results are summarized in Table 13.

Table 13

Operation of Units Under Long Term Exposure to Sodium

<u>Unit</u>	<u>Effluent Acetic Acid</u>	<u>Efficiency</u>
Control	100 mg/l	99.5
2000 mg/l	300 mg/l	98.0
3000 mg/l	500 mg/l	96.75
4000 mg/l	800 mg/l	94.75
6000 mg/l	1500 mg/l	90.0

On the fifty-third day of this run 0.011 M potassium was added to the 6000 mg/l unit, and maintained at this concentration for the remainder of the study. This produced an immediate significant increase in the rate of acetate utilization (from 90% to 95%). On the eighty-fifth day 0.0085 M potassium was added to the 4000 mg/l unit. This procedure again produced a significant increase in the efficiency of acetate utilization. The addition of the potassium antagonist in the two instances above was performed to determine whether antagonism would be accomplished even after acclimation had taken place. The results clearly indicate that even when the bacteria had been subjected to sodium for a sufficient period to allow acclimation to take place, antagonism would still be manifest.

In addition to following the operation of these units by the volatile acid analysis, periodic measurement of the mixed liquor organic nitrogen concentration was performed as an indication of the magnitude of biological synthesis taking place in the units. The results of these measurements are shown in Figures 7 and 8. Both the operational efficiency of the units and the ratio of organic nitrogen in each unit to that in the control are shown plotted versus time in these figures. It can be seen that the organic nitrogen ratio decreased faster than and reached much lower values than the operational efficiency for each unit. Even the unit containing 2000 mg/l of sodium which operated throughout the study at an efficiency of 98% exhibited an organic nitrogen concentration which was 73% of that of the control by the last day of the study. The results clearly indicate that sodium acts to reduce bacterial synthesis even at concentrations below those at which it has a significant effect on the rate of substrate utilization.

The introduction of potassium to the 4000 mg/l and 6000 mg/l sodium units resulted in an immediate increase in the organic nitrogen concentration. By the completion of this run the organic nitrogen concentration in these units was such that the ratio of the organic nitrogen in each unit to the control value equalled the operational efficiency of each unit. Thus the action of the potassium antagonist was to completely eliminate the synthesis reduction.

DISCUSSION OF RESULTS

a) Confirmation of Slug Feed Study Results

A summary of the results of runs 1-4 with reference to the results of the slug feed studies (1) is given below.

1. In all cases tested, antagonism between cations which were evidenced in the slug feed runs were confirmed in the daily feed runs.
2. In all cases where the slug feed studies indicated antagonism by a combination of cations to be superior to that achieved by a single antagonist, such was the case in the daily feed studies.
3. The slug feed studies indicated that in many instances the presence of antagonists would produce a stimulatory effect with respect to the control even in the presence of a high concentration of the toxic cation. This phenomenon was observed in the daily feed runs.
4. The phenomenon of secondary antagonism was observed in several instances in the slug feed runs. In run 4 in this study, the only run in which this phenomenon was tested, secondary antagonism was achieved.

It is clear from the results reviewed above that the phenomena and relationships established in the slug feed studies should hold substantially under continuous exposure conditions. To be sure not all of the phenomena and only a few of the relationships found in the slug feed studies were tested. However, in every case tested, confirmation was obtained.

b) Acclimation

The results reported by previous investigators which were reviewed earlier indicated that substantial reduction in the toxic effect of a cation could be manifest through the process of acclimation. A comparison between the results of the slug feed studies (1) and the results of runs 1-4 of this investigation confirm this expectation. This is illustrated in Figure 9 in which

the acetate utilization rates with respect to the control for the slug and daily feed studies is plotted versus the concentration of each cation. Figure 9 indicates that the tolerance level to the cations studied can be increased about 2 to 3 times by acclimation. It is possible that a greater degree of acclimation could be obtained if the rate of increase of the toxic cation concentration was slower than the rates used in this study.

c) Acclimation Plus Antagonism

The results reported previously 1) and in this study indicate that the two major phenomena which can be utilized to mitigate the toxic effect of any cation are antagonism and acclimation. It should be expected that a combination of both phenomena would produce results superior to either one alone. Runs 1-4 seem to confirm this expectation as greater efficiency of acetate utilization was always obtained with antagonists present. However, these runs do not yield conclusive proof as the antagonists were present from the start of the runs. It could thus be argued that in the units containing the antagonists, acclimation did not take place as it was not required of the organisms to combat the toxicity.

Run 5 yields more conclusive proof that both acclimation and antagonism will take place simultaneously. In this run the sodium concentration was slowly raised over a rather long period of time (53 to 85 days) prior to the addition of any antagonist. The addition of potassium even after a sufficient period for acclimation was provided, produced a significant increase in the efficiency of acetate utilization.

d) Enzyme Mechanism for Acclimation

At the present time the most widely held theory of cation toxicity is that the toxic cation damages or inactivates some of the critical enzymes of the organism. Based on his studies of acclimation to drug toxicity, Hinshelwood (10) postulated that the process of acclimation involves a readjustment of the enzyme balance in the cell. The object of the readjustment being to provide extra enzymes to the section of the cell where metabolism is inhibited. The result is either a replacement of the damaged enzyme, the strengthening of an existing alternate metabolic pathway, or the construction of a shunt pathway around the inhibited area. In essence, acclimation involves a strain on the resources of the organism. As the organism has only a certain reserve capacity which it can marshal, unlimited acclimation is impossible.

It is thus not surprising that acclimation and antagonism can take place simultaneously, and that a combination of these effects produces results superior to that achieved by either process alone. Antagonism can function in two ways to aid acclimation. First it can reduce the strain on the resources of the organism by

overcoming a portion of the inhibitive effects of the toxic cation. Thus the organism can deal with metabolic areas of the cell where the antagonistic cation cannot act. In addition, the presence of an antagonist can protect the cell from irreparable injury during the time lag involved before acclimation can take place.

e) Sodium Synthesis Block

In run 5 it was observed that in the presence of moderate levels of sodium, synthesis of the methane bacteria was blocked independently of the ability of the organism to degrade the substrate. Thus some of the energy released in the degradation of the acetate was not or could not be used for synthesis.

The ability of potassium to eliminate this synthesis block provides a clue to the possible mechanism of this effect. Dugid and Wilkinson (11) reported that carbohydrate synthesis in *A. aerogenes* was inhibited by a deficiency of potassium. Several biologists have reported that cells tend to accumulate potassium and excrete sodium against a concentration gradient (12)(13)(14). Rothstein (14) postulated that the cell operated as an ion pump on the energy released from metabolism of the substrate to drive potassium against a concentration gradient and thus maintain the proper internal potassium concentration. Actually, the ion pump is only an artifice; the actual mechanism postulated is a utilization of energy released in metabolism to carry out an active transport of cations across the cell membrane.

The ion pump mechanism gives a good qualitative explanation of the sodium produced synthesis block. The imbalance in the sodium-potassium levels produced in the cell could force the ion pump to operate at a very high load in order to maintain a sufficient potassium level in the cell. This extra energy could only be taken from that released in the breakdown of the substrate, which would consequently reduce the energy available for synthesis. The introduction of potassium into the medium would then decrease the concentration gradient which the pump is forced to operate against and thus decrease the pump energy requirement.

The ability of sodium to block synthesis without interfering with substrate utilization is significant in establishing criteria for laboratory scale digestion studies. For example, a short time test of the treatability of a waste would not be appropriate if sodium was present. In a short time test the washout of organisms which would result from the synthesis block would not be sufficient to produce adverse results. Thus a waste could appear treatable from laboratory studies, but after a long period of field operation failure would eventually result.

From the point of view of full scale digester operation, it would be good practice to add enough potassium to prevent the synthesis block in any waste containing high concentrations of sodium.

f) Allowable Cation Concentration for Waste Treatment

The fundamental purpose of these studies into the effects of cations on the anaerobic waste treatment process is to provide the sanitary engineer with data which can be used in the design of waste treatment systems. One of the major design criteria required is the upper limit of cation concentration. This standard is defined as the maximum concentration of a cation which can be present in a waste without interfering with adequate operation of the unit. Values of this criteria were previously presented for slug addition of the cation (1). In this study values were obtained for daily feed systems. The values for both slug and daily feed systems with and without antagonists present is shown in Table 14.

Table 14

Upper Limit of Cation Concentration

Cation	Slug Feed		Daily Feed	
	Single Cation M/l	Antagonists Present M/l	Single Cation M/l	Antagonists Present M/l
Na	0.2	0.3 - 0.35	0.30	>0.35
K	0.90	0.15 - 0.2	0.13	>0.35
Ca	0.07	0.125 - 0.15	0.15	>0.20
Mg	0.05	0.125	0.065	>0.14

A comparison of the values presented in Table 14 indicates the marked effect of antagonism and acclimation in reducing cation toxicity.

The values presented in Table 14 are strictly applicable only at the organic loading and biological solids retention time used in these studies. It is to be expected that as the organic loading increases, the upper limit of cation concentration would decrease. This is so because in two units operating at different organic loadings, the unit at the higher loading must function at a higher efficiency if both units are to produce an effluent of the same quality. For example, if a unit is loaded at one gram per liter per day of acetic acid at a 15 day hydraulic detention time and produces an effluent with 1500 mg/l of volatile acids, the efficiency of operation is 90%. In order for a unit at the same hydraulic detention time to produce the same effluent quality at a loading of 2 grams per liter per day the required efficiency is 95%. Thus if a cation concentration produces a certain percentage inhibition of the rate of substrate utilization, failure is more likely in the more heavily loaded unit. This point is illustrated by the results of runs 1 and 5. In run 1 with an organic loading of 0.5 grams per

liter per day, the effluent volatile acids from unit #2 was 850 mg/l when the sodium concentration reached 0.26 M. This is equivalent to an operational efficiency of 89%. In run 5 the unit receiving 0.26 M sodium exhibited an effluent volatile acids concentration of 1500 mg/l. This is equivalent to an efficiency of 90%. Thus although both units exhibited essentially the same operational efficiency, the more heavily loaded unit had a volatile acid concentration more nearly approaching the bicarbonate buffer concentration and so was nearer to low pH failure.

It should be expected that a decrease in the biological solids retention time would also reduce the upper limit of cation concentration, the lower the biological solids retention time, the greater the strain on the organisms to resynthesize to the proper level after the daily solids wasting. Thus the effect of a toxic material would be magnified in the unit with the low biological solids retention time because the organisms present would be required to metabolize the waste at a higher rate per organism than in a unit with longer biological solids retention time.

CONCLUSIONS

1. The phenomena and relationships observed in slug feed studies for cation effects are applicable to daily feed operation.
2. Acclimation to the toxic effect of a cation will take place under daily feed operation.
3. Acclimation increases the tolerance level to the toxic effect of a cation two to three times.
4. The phenomena of acclimation and antagonism can take place simultaneously. When both are called into play, cation toxicity is alleviated to a greater extent than by either process alone.
5. Sodium produces a significant synthesis block at concentrations below those at which it will affect the rate of acetate utilization.
6. At the organic loading of 0.5 grams per liter per day of acetate, and at a 15 day retention time, the upper limit of cation concentration with no antagonists present is 0.3 M for sodium, 0.13 M for potassium, 0.15 M for calcium and 0.065 M for magnesium.
7. Under the same conditions but with antagonists present, the upper limit of cation concentration was not exceeded in this study, but was greater than the following: 0.35 M for sodium, 0.35 M for potassium, 0.20 M for calcium and 0.14 M for magnesium.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service Research Grant WP-482, as well as Research Fellowship 6F-13, 105, both from the Division of Water Supply and Pollution Control, Public Health Service.

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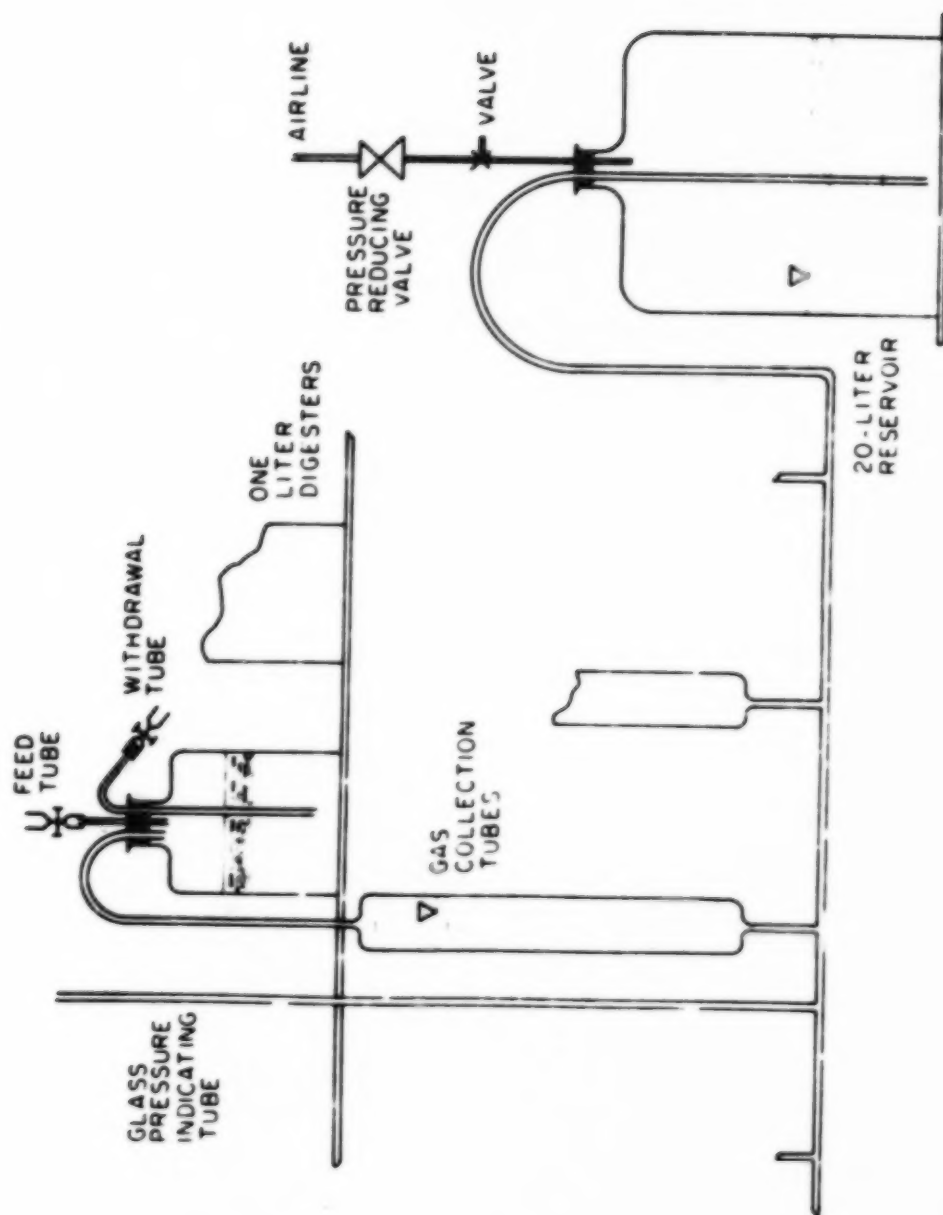


FIGURE 1. DAILY FEED DIGESTER SYSTEM

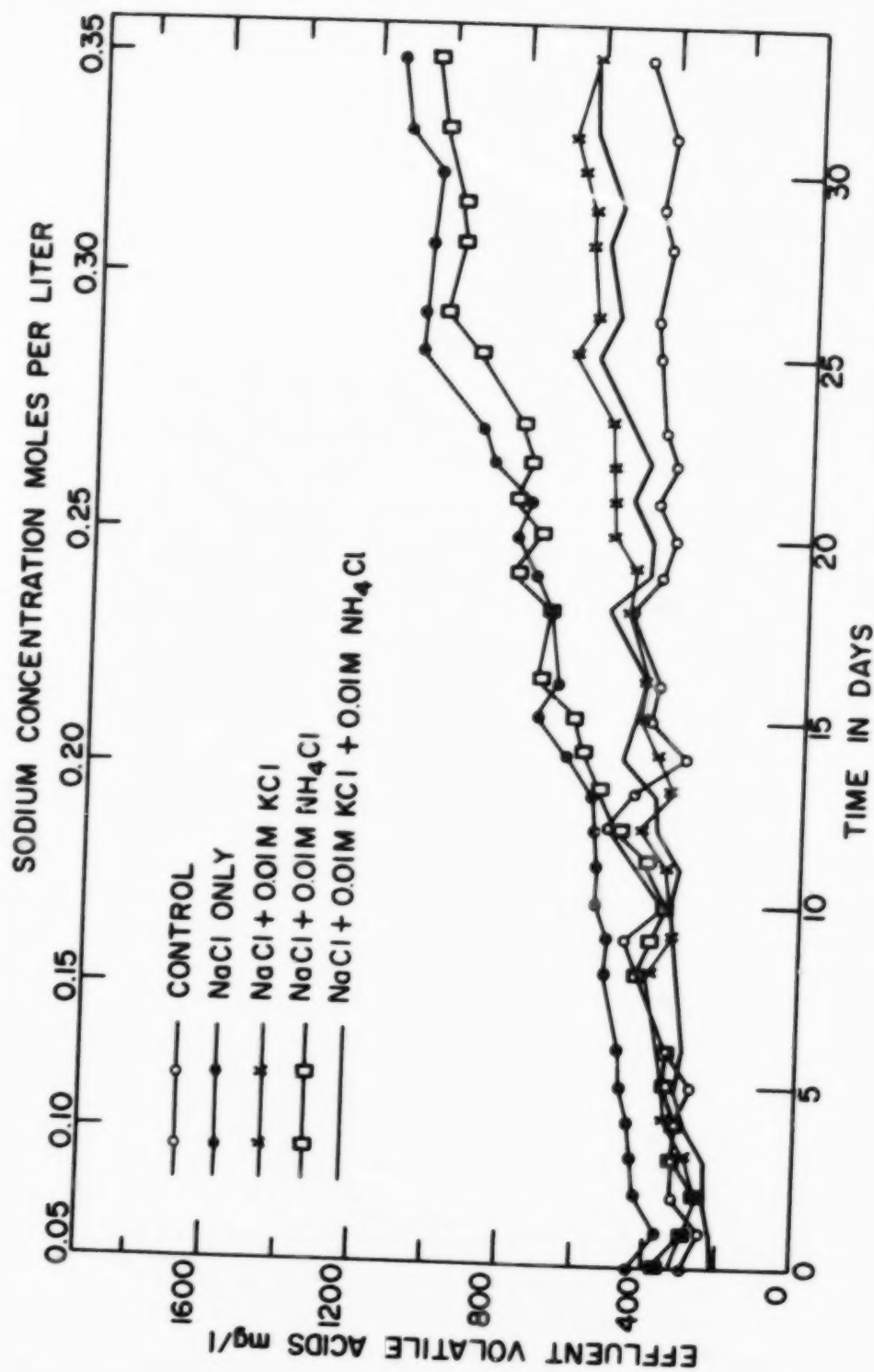


FIGURE 2. SODIUM TOXICITY - DAILY FEED

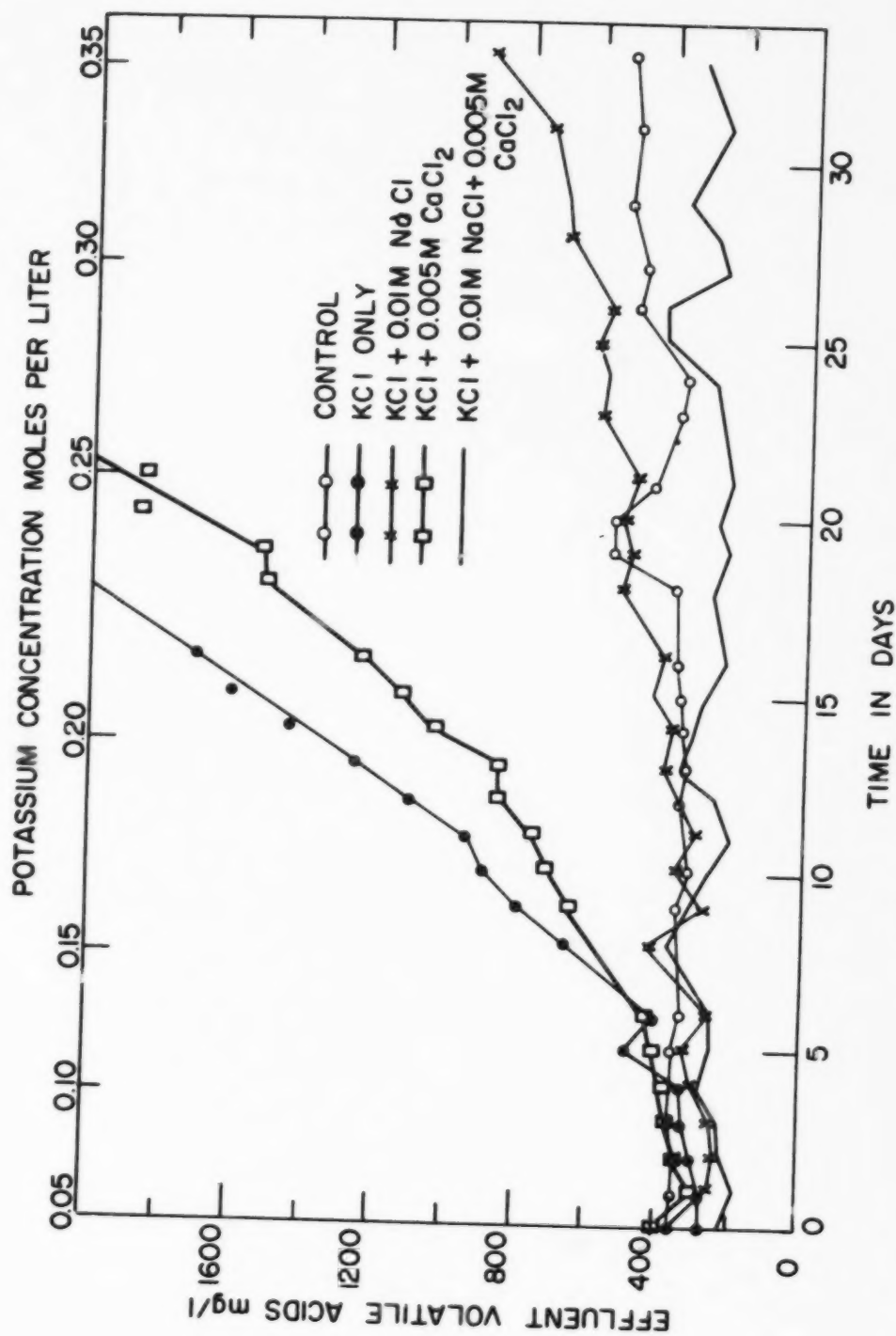


FIGURE 3. POTASSIUM TOXICITY - DAILY FEED

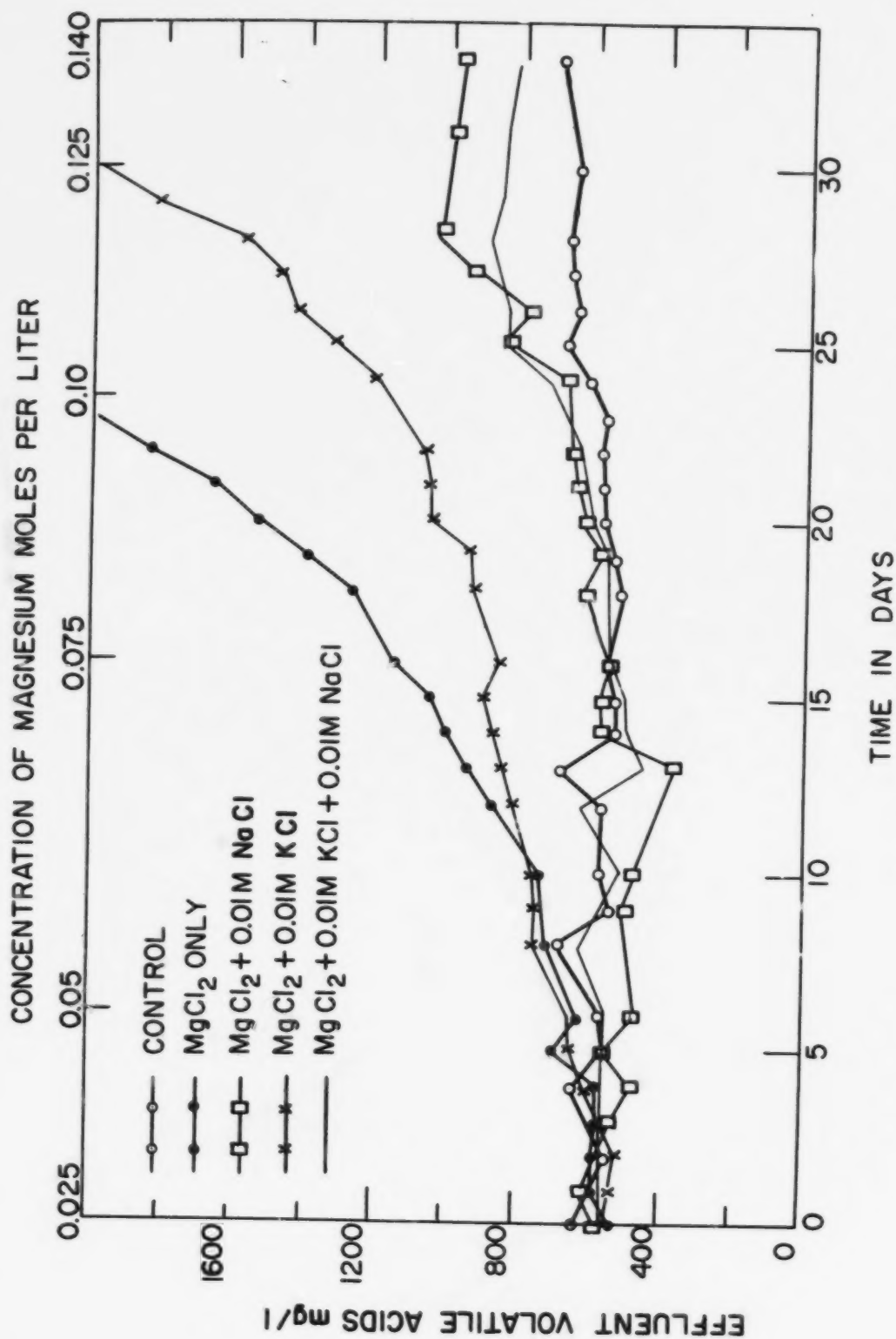


FIGURE 4. MAGNESIUM TOXICITY - DAILY FEED

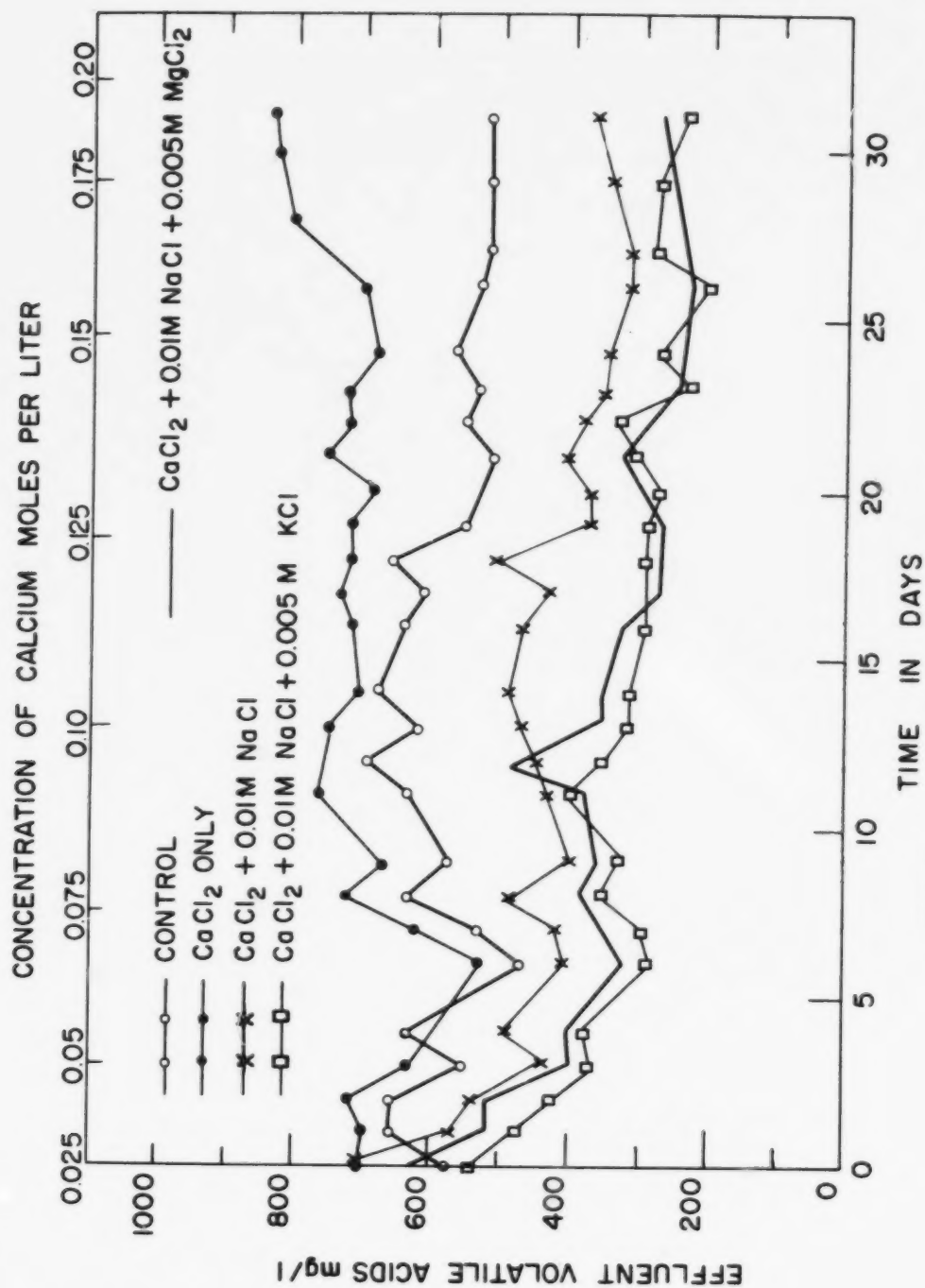


FIGURE 5. CALCIUM TOXICITY - DAILY FEED

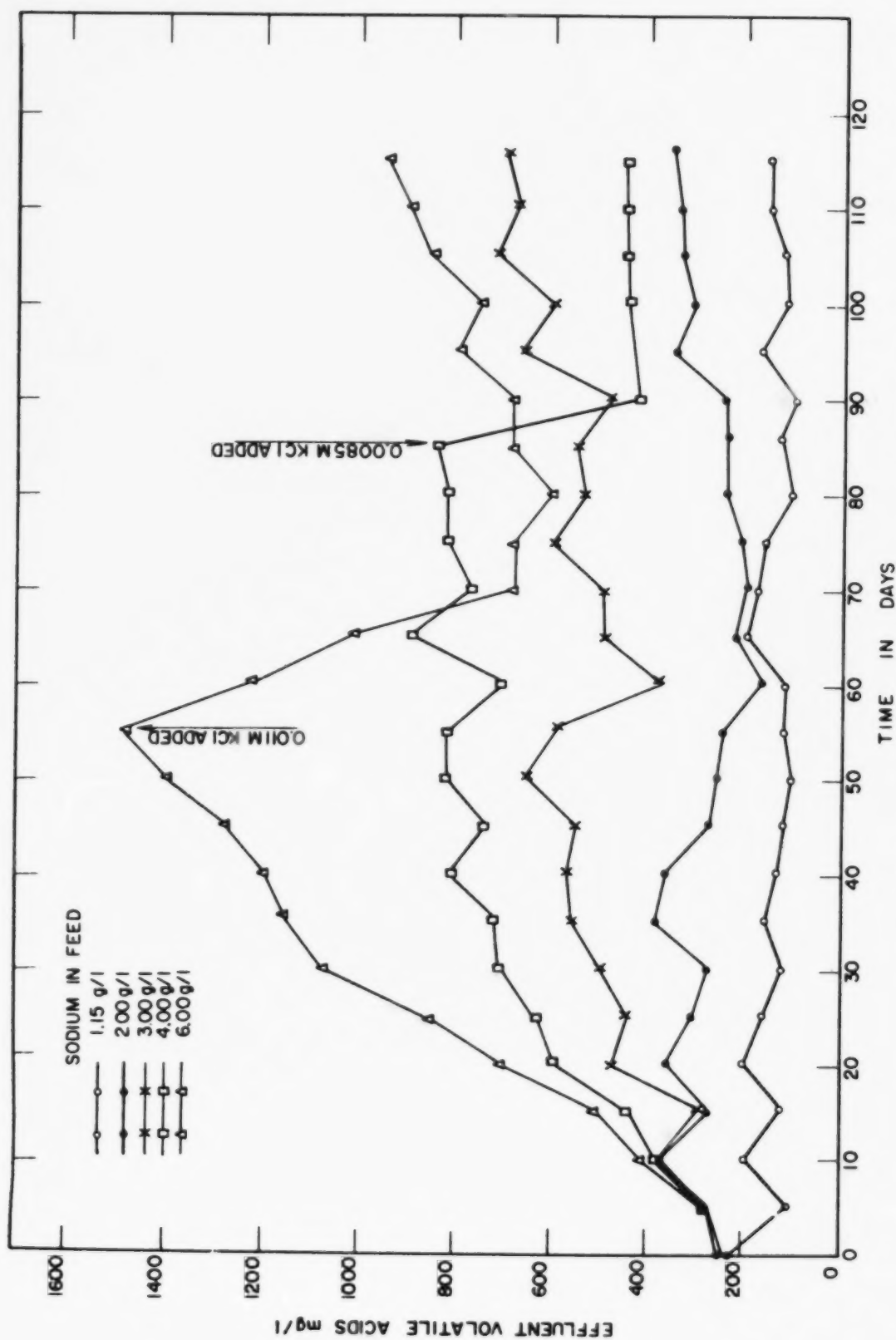


FIGURE 6. OPERATION OF DAILY FEED UNITS - RUN 5

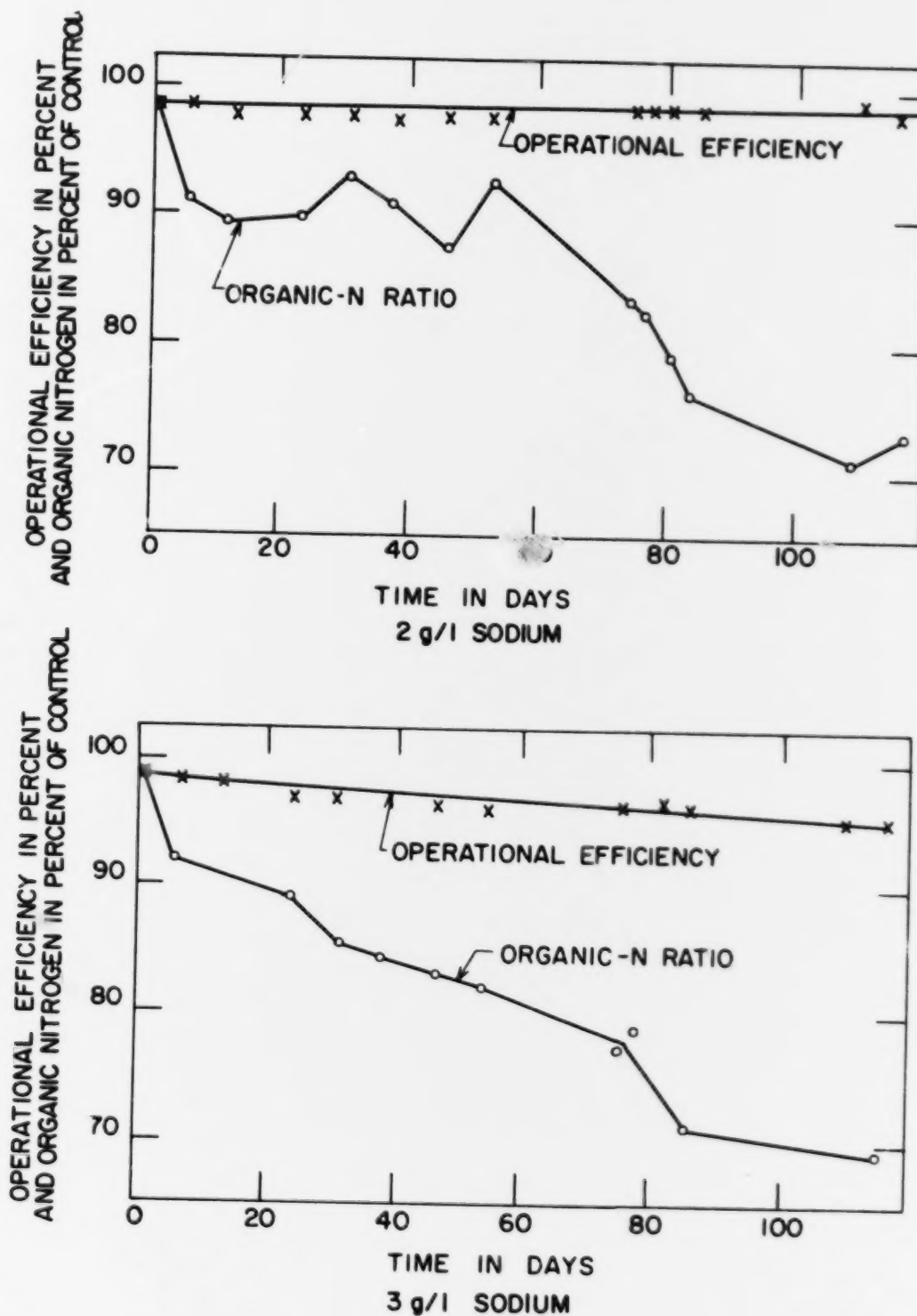


FIGURE 7. OPERATIONAL EFFICIENCY AND ORGANIC NITROGEN
RATIO - 2 g/l AND 3 g/l SODIUM UNITS - RUN 5

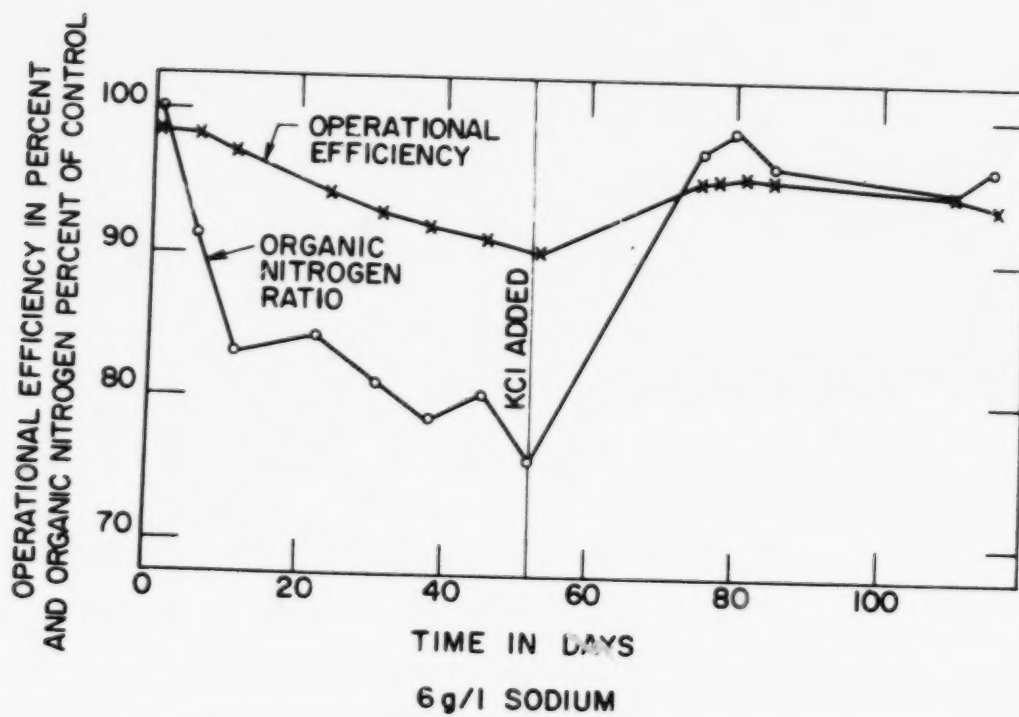
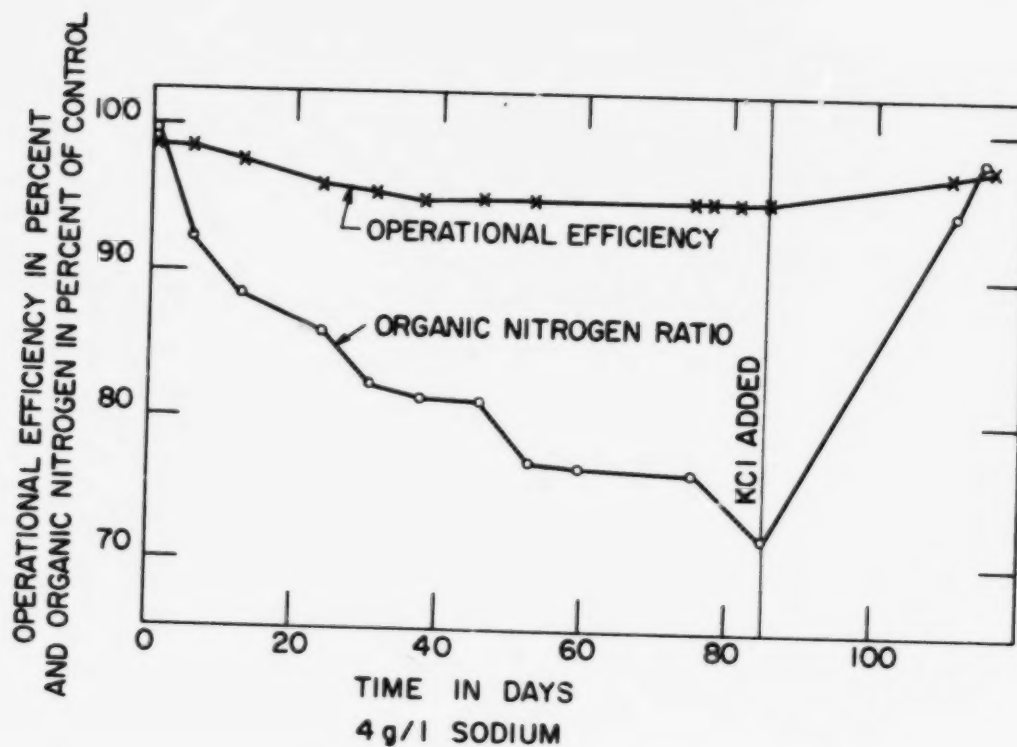


FIGURE 8. OPERATIONAL EFFICIENCY AND ORGANIC NITROGEN
RATIO - 4 g/l AND 6 g/l SODIUM UNITS - RUN 5

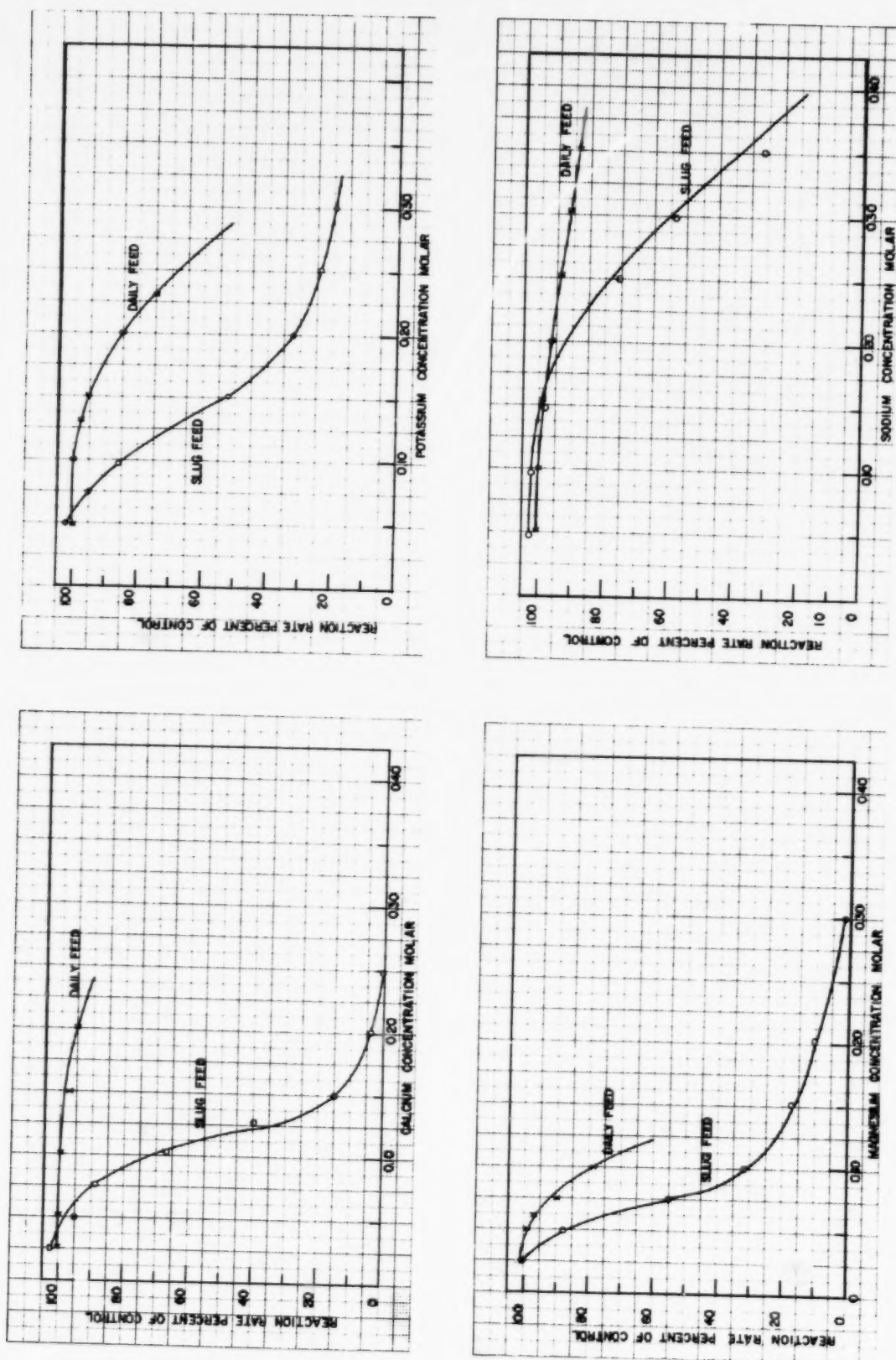


FIGURE 9. COMPARISON BETWEEN TOXICITY OF IONS FROM
DAILY FEED AND SLUG FEED STUDIES